TESTS FOR BACILLUS COLI AS AN INDICATOR OF WATER POLLUTION

By C.-E. A. Winslow

For nearly twenty-five years the number of colon bacilli present in water has been used as a test of its sanitary quality. The writer does not know who originally devised this test; but the first instance of its use with which he is familiar in this country is the study by Theobald Smith of certain New York river waters in 1893.[1]

The value of the colon test has been more and more generally recognized in this country and in England. In Germany there has always been a strong school which has been doubtful of its significance, but the more important recent papers, such as that of Quantz,[2] recognize the value of the colon test if intelligently applied. The German investigators have performed a valuable service in emphasizing the fact that the number of colon bacilli present in a water is only one link in a chain of circumstantial evidence of which the sanitary inspection forms an essential and integral part. The writer is also wholly in accord with their contention that the 20° gelatin count is a test which is often of the greatest assistance in forming judgment of the quality of a water, having in mind for example the case of the water supply of Auburn, New York, Lake Owasco. In this lake, according to the exhaustive data collected by Mr. J. W. Ackerman the gelatin count rises sharply each year at the time of the spring thaw, while colon bacilli, never very abundant, are more numerous during the summer.

The explanation of this phenomenon probably is that a certain proportion of colon bacilli are always contributed by the small brooks which enter the lake from agricultural land, which, not being of human origin, have but little significance. At the time of the spring thaws, which for the most part wash an open farming country, the normal contribution of *B. coli* from the fields is obscured by the rain and melting snow, while only the rise in total count

[1] Thirteenth Ann. Rep. State Bd. Health of N. Y. for 1892, p. 712.

[2] *Zeit. f. Hyg.*, 1914, vol. lxxviii, p. 193.

registers the fact that contaminating material of all sorts is being washed into the lake. With this contaminating material, for the most part of a harmless nature, human excreta is washed in from certain points on the watershed, and in the spring of 1908 the excreta contained specific typhoid infection and an epidemic in Auburn was the result. In this case then the total count of bacteria was a more accurate index of danger than the colon content. Fully granting, however, the importance of sanitary inspection and the value of the gelatin count it remains true that the colon test is one of the most valuable diagnostic instruments at the disposal of the sanitarian.

There can be no doubt that colon bacilli are typical inhabitants of the intestine of man and other warm blooded animals; that these intestinal colon bacilli tend to die out rather rapidly in water; and that they are not found in very large numbers in any natural waters not subject to considerable pollution from sewage or recent wash from the surface of the soil. The writer sees no reason to modify the conclusion of Professor Prescott and himself that:

> Altogether the evidence is quite conclusive that the absence of *B. coli* demonstrates the harmlessness of a water as far as bacteriology can prove it. That when present, its members form a reasonably close index of the amount of pollution, the authors above quoted have proved beyond reasonable cavil. It may safely be said that when the colon bacillus is found in such abundance as to be isolated in a large proportion of cases from 1 cc. of water, it is generally proof of the presence of serious pollution.

The colon group may be broadly defined as including all aerobic non-spore-forming bacilli which produce acid and gas in glucose and lactose media. This is a large and complex group, and it is of great importance to determine whether all of its members are equally significant, or whether there are special types which are more intimately associated with the intestines of warm blooded animals and hence of special importance from a sanitary standpoint. The group as a whole is not present in very large numbers in pure waters, but in order to obtain results of any precision it is important to define the intestinal *B. coli* if possible more closely.

Bacteriologists in the laboratories of the Massachusetts State Board of Health[3] and the Massachusetts Institute of Technology long ago attacked this problem and their conclusions were embodied

[3] Thirtieth Ann. Rep. State Bd. Health of Mass. for 1898, p. 533.

in the 1905 Report of the Committee on Standard Methods of Water Analysis of the American Public Health Association.[4] The identification of the colon group involved the determination of typical morphology, motility, fermentation of glucose broth, coagulation of milk, production of indol and reduction of nitrates, all under definitely standardized conditions.

These tests were pretty generally adopted in this country for the next five years after the committee on standard methods made its report. Gradually, however, practical laboratory men became restive under the exactions of this procedure. The complete test required the use of seven different media and took nine days to complete. Furthermore there was no definite evidence that the tests used were really calculated to distinguish between fresh fecal colon bacilli and others, the only adequate basis for such a time-consuming and troublesome procedure. There is to-day not the slightest reason to suppose that motility and nitrate reduction at least have any important significance of this kind.

When therefore Whipple[5] suggested the use of a single "presumptive test" gas production in glucose broth, and particularly when Jackson[6] introduced the new test of gas production in lactose bile, they received a ready hearing among bacteriologists. It was known that lactose bile would inhibit a certain number of sensitive strains of *B. coli*, particularly in waters of fairly good quality, while on the other hand it was recognized that other organisms than colon bacilli, particularly anaerobic spore formers, would at times give fallacious positive results. It was shown, however, that in a number of waters studied this error was not a large one, and since the colon test can only be interpreted broadly in any case it was generally believed that the slightly greater accuracy of the older isolation tests scarcely compensated for the trouble involved. The laboratory section of the American Public Health Association, at its Washington meeting, in September 1912, adopted a resolution recommending determinations of counts at 20° and 37°, and the lactose bile presumptive test as the standard routine procedure in water examinations.

Experience obtained during the past two years has, however,

[4] *Jour. Infect. Dis.*, 1905, Supp. No. 1, p. 1.

[5] Tech. Quart., 1903, vol. xvi, p. 18.

[6] Biological Studies by the pupils of William Thompson Sedgwick, 1906; also *Jour. Inf. Dis.*, 1907, Supp. No. 3, p. 30.

tended to emphasize the importance of the error involved in the assumption that gas formation in lactose bile is even a rough index of the presence of bacilli of the colon group. It appears that, while a large percentage of random bile tests on waters of various origin may prove to be colon bacilli, certain waters, and particularly those of rather good quality, may consistently give the bile presumptive test without colon bacilli being present at all. George W. Fuller[7] has recently pointed out a number of instances of this kind. The water supply of Cincinnati, for example, when drawn from a highly polluted part of the river showed colon bacilli, as determined by confirmatory tests, in 60 per cent of 1 cc. samples, while at the present intake seven miles above and in a vastly better location it shows positive presumptive tests in 85 to 90 per cent of the samples tested. In New Orleans complete confirmatory tests used to show colon bacilli only 3 per cent of the time when from 100 to 300 cc. of water were tested, while presumptive tests in 1 cc. are positive in 80 per cent of the samples tested now. At Columbus, Ohio, Grand Rapids, Michigan, and Evanston, Illinois, Fuller cites cases of well filtered and almost sterile waters giving positive presumptive tests.

The International Joint Commission on the Pollution of Boundary Waters presented striking evidence along this line cited in Table 1.

The most exhaustive investigation of this point is the admirable study of the Potomac River by Hugh S. Cumming of the United States Public Health Service.[8] The principal results of this study, so far as they bear on the presumptive test are cited in Table 2.

It is evident from this table that in the upper reaches of the river where colon bacilli were present to the number of 100 or more per cubic centimeter the majority of the gas formers were *B. coli.* At Maryland Point, however, and below, where the pollution was much less, one to two-thirds of the gas formers present were not colon bacilli at all. Anaerobic spore forming bacilli of the *B. Welchii* or *B. sporogenes* type were isolated from the tubes which gave gas but no *B. coli,* and a special study by inoculating fermentation tubes at 70° F. showed that these gas forming anaerobes were quite constantly present in numbers varying from 1 to 50 per 10 cc. of water.

In a recent series of examinations of water from the five different sources which supply the city of New Haven, a considerable percent-

[7] *Jour. Franklin Inst.,* 1915, vol. clxxx, p. 17.

[8] Bull. 104 U. S. Hyg. Lab., 1916.

age of the samples showed gas formers, with no colon bacilli present, the positive gas tubes failing to show any fermenting organisms at all when plated out on lactose agar plates.

TABLE 1

Gas producers, St. Johns River

(International Joint Commission on the Pollution of Boundary Water)

SAMPLING POINT	NUMBER OF SAMPLES TAKEN	AVERAGE PER 100 CC. BY PHELPS METHOD		
		Total gas producers	Typical B. coli	Anaerobic gas producers
1	21	467	65	402
2	21	647	160	487
3	21	566	70	496
4	20	472	167	305
5	21	536	160	376
6	21	643	112	531
7	21	515	155	360
8	21	730	250	480
9	21	433	18	415
10	21	433	112	321
11	21	519	57	462
12	21	600	61	539
13	21	519	74	445
14	21	561	103	458
15	21	566	164	402
16	21	604	155	449
17	21	476	31	445
18	21	480	117	363
19	21	467	117	350
20	21	600	155	445
21	21	520	103	417
22	21	566	112	454
23	21	1,086	725	361
24	21	695	202	493
25	21	614	112	502
26	21	601	117	484
27	21	524	57	467
28	20	540	254	286
29	21	1,090	631	359
30	20	476	112	364
31	21	480	112	364
32	21	528	70	458

With disinfected waters the error due to the presence of the anaerobic spore bearers is of course particularly serious, as noted by Wells at Grand Forks, North Dakota, by Palmer at Trenton, New

TABLE 2

Gas-forming organisms from various stations confirmed or not as being B. coli

(Potomac River, Hugh S. Cumming)

CROSS SECTIONS	NUMBER OF SAMPLES	AVERAGE NUMBER OF B. COLI PER CC.	AVERAGE NUMBER OF GAS FORMERS NOT B. COLI PER CC.	B. COLI, PERCENTAGE OF TOTAL GAS FORMERS
Giesboro Point	770	295.0	24.1	92.4
Fort Foote	789	254.0	9.7	96.3
Fort Washington	778	123.0	6.2	95.2
Mount Vernon to Whitestone Point	851	102.0	8.5	92.3
Indianhead	241	123.0	15.7	88.6
Possum Point	212	66.2	13.8	82.7
Maryland Point	691	1.44	0.75	65.7
Popes Creek	740	0.19	0.33	36.5
Lower Cedar Point	476	0.14	0.16	46.6
Below Lower Cedar Point	2,261	0.052	0.057	47.5

Jersey and others. Altogether Dr. Cumming is certainly right in his conclusion that:

> The reliability of the lactose-broth and lactose-bile presumptive test varies directly with the degree of pollution, therefore inversely with the remoteness in time and distance from the source of pollution. This is due to the general occurrence of a group of organisms in small and almost constant numbers, approximating 1 per cc., which are not manifest when the number of *B. coli* is large, but appear evident when the number of *B. coli* approaches or is less than 1 per cc.

The presumptive test must then certainly be abandoned except for waters of very bad quality, or at least supplemented by confirmatory plating to show the presence of the aerobic non-spore forming colon group.

Granting that such confirmatory tests must be made, the question is still open whether lactose bile shall still be used for preliminary enrichment of the sample, or whether lactose broth or some other medium may not be preferable if the idea of a single presumptive test is to be abandoned. It has been generally acknowledged that the use of lactose broth followed by confirmatory tests would give a larger number of positive results than the use of lactose bile followed by confirmatory tests, since bile inhibits a certain proportion of organisms of the colon group. The only real advantage lay in its usefulness as a single presumptive test because of the fact that lac-

tose broth gives much too high results if used as a presumptive test alone. Thus Stokes and Stoner[9] found that of cultures isolated from tubes showing gas formation in lactose broth and lactose bile 88 per cent and 95 per cent respectively proved to be *B. coli;* so Cumming reports 70 per cent of positive broth tubes and 87 per cent of positive bile tubes confirmed in the Potomac River. If confirmatory tests are to be made, however, they will take care of any errors due to the formation of gas by organisms other than *B. coli*, while the absolutely higher results obtained with lactose broth offer a very important advantage. Thus in Dr. Cummings' study of the Potomac River, to which reference has been made, the total number of colon bacilli per cubic centimeter as determined by lactose broth followed by confirmatory tests was 84 against 47 for lactose bile followed by confirmatory tests. M. M. Obst[10] has recently compared lactose bile and lactose broth as preliminary enrichment media, coming to the same conclusion, that bile inhibits about half the colon bacilli which may be obtained by the use of lactose broth. Mrs. Obst also emphasizes the costly and laborious procedure often involved in obtaining the bile medium.

Altogether it may be concluded that the method of testing for the colon group which has been adopted by the United States Hygienic Laboratory probably represents the best present practice, a practice which the writer personally hopes will be adopted by the Committee of the American Public Health Association on Standard Methods for the Bacteriological Examination of Water when it finally makes its long-expected report. The essential points in this method are (a) preliminary enrichment of the sample to be tested in lactose pepton broth for 48 hours at 37°C.; (b) plating on Endo medium or lactose litmus agar from all samples showing gas after 24 hours and again after 48 hours from those tubes which have become positive after being negative the first time; (c) in case plates are doubtful fishing from suspicious colonies to a confirmatory fermentation tube of lactose broth, which if positive should show 10 per cent or more of gas after 48 hours at 37°.

Accepting this test as a simple and accurate method of determining the presence of bacilli of the colon group taken as a whole there still remains the important question whether any further tests can, with advantage, be used to distinguish special types within the group

[9] *Amer. Jour. Pub. Hyg.*, vol. xix, 1909, p. 312.
[10] *Jour. Bact.*, vol. 1, 1916, p. 73.

which may be of particular importance as indicators of recent fecal pollution.

Many observers, both in this country and in England, have pointed out that in recently polluted waters the bacilli of the colon group are apt to be of a more vigorous type, fermenting sugars and producing indol in a higher percentage of cases. Thus Houston[11] showed that 53 per cent of the strains isolated from raw river waters were "typical" in fermenting lactose and forming indol against only 34 per cent of the strains found in stored and filtered waters. The most marked differences appeared in the fermentation of saccharose and raffinose and the production of indol.

In the attempt to classify bacilli of the colon group by their fermentative reactions saccharose has usually been considered as of primary importance. All of the members of the group of course ferment glucose and lactose, but as pointed out by Theobald Smith[12] saccharose fermentation divides the series into two more or less distinct types. MacConkey[13] followed by Jackson[14] and the Committee on Standard Methods have further subdivided the positive and negative saccharose groups according to the fermentation of dulcite. The table below indicates the general characters of the four types of the colon group as they have most commonly been defined.

TABLE 3

FERMENTATION OF		TYPE
Saccharose	Dulcite	
+	−	B. aerogenes
+	+	B. communior
−	+	B. communis
−	−	B. acidi-lactici

Kligler[15] believes from his studies that salicin fermentation is more closely correlated with other characters than is the fermentation of dulcite. He distinguishes *B. aerogenes* from *B. communior* by positive fermentation of salicin rather than negative fermentation

[11] Seventh Report on Research Work, Metropolitan Water Board, London, 1911.

[12] The Wilder Quarter-Century Book, 1893, p. 187.

[13] *Jour. Hyg.*, vol. v, 1905, p. 333; also *Jour. Hyg.*, vol. vi, 1906, p. 385.

[14] *Jour. of the Am. Pub. Health Assn.*, vol. i, p. 930.

[15] *Jour. Inf. Dis.*, 1914, vol. xv, p. 187.

of dulcite, and *B. acidi-lactici* from *B. communis* by negative fermentation of salicin rather than negative fermentation of dulcite.

The difficulty inherent in such classifications as the above is that they are in large measure arbitrary. One can make an almost endless number of divisions by using different fermentable substances, and they cross each other in the most confusing manner. Furthermore the attempt to show any constant relation between the MacConkey types and the quality of the water in which they occur has not proved successful. Clemesha[16] found in a study of surface waters in India that bacilli of the *B. communis* and *B. communior* (dulcite positive) types were associated with fresh pollution, while those allied to *B. acidi-lactici* and *B. aerogenes* were more persistent in stored waters; but Houston[17] in England failed to confirm these conclusions. Two years ago it seemed that the attempt to work out a classification of the colon group which would be of practical sanitary value was almost hopeless.

A very interesting discovery of Harden and Walpole's[18] has recently been developed by Rogers, Clark and Davis of the United States Bureau of Animal Industry[19] into the most important step taken for twenty years in the classification of the colon group. These observers find that the fermentation of glucose by bacteria of the colon series may follow two entirely different lines, leading in one case to the production of gas which when collected under a vacuum is composed of about equal parts of carbon dioxid and hydrogen, while in the other case the ratio of carbon dioxid to hydrogen is in the ratio of 2 to 1. The second or high ratio group is usually saccharose-positive and dulcite-negative, corresponding to the older definition of *B. aerogenes*.

As long as the differentiation of this peculiar high-ratio type depended on gas analysis conducted in a vacuum it was of little practical importance as a basis for routine procedure. Clark and Lubs,[20] however, have correlated the high ratio type of fermentation with

[16] The Bacteriology of Surface Waters in the Tropics, Calcutta, 1912.

[17] Seventh Report on Research Work, Metropolitan Water Board, London, 1911.

[18] *Jour. of Hyg.*, 1905, vol. v, p. 488; also *Proc. Roy. Soc.* (B), 1905–1906, vol. lxxvii, p. 399.

[19] *Jour. Infect. Dis.*, 1914, vol. xiv, p. 411; also *Jour. Infect. Dis.*, 1914, vol. xv, p. 100.

[20] *Jour. Infect. Dis.*, 1915, vol. xvii, p. 160; also *Jour. of Biol. Chem.*, 1915, vol. xxii, p. 87.

the hydrogen ion concentration in glucose broth and have thus given us a simple and reliable practical test for its recognition.

Rogers, Clark and Lubs have also performed a service of capital importance in calling attention to the serious errors involved in the ordinary methods of titrating media by adding strong acid or alkali to bring the reaction to a definite point. The true hydrogen ion concentration can be determined only by the use of the potentiometer or by the application of a graded series of indicators such as have been worked out by Lubs and Clark.[21] The use of these methods shows a striking difference between the colon and the aerogenes types as indicated by the table below from results recently obtained by the colorimetric method in the writer's laboratory at the Yale Medical School.

Rogers and his associates pointed out that the high-ratio, low-hydrogen-ion aerogenes strains are usually saccharose-positive, raffinose-positive, and dulcite-negative, and very often indol-negative, and that among them are to be found representatives of the liquefying or *B. cloacae* type. Winslow and Kligler[22] have specially emphasized the negative indol reaction of *B. aerogenes*. Levine[23] following Harden and Walpole,[23] Harden[24] and Harden and Norris,[25] has shown that a positive Voges-Proskauer reaction is also characteristic of this type.

The most important point, however, from a practical sanitary standpoint is the origin in nature of these two sub-types of the colon group. It was the discovery by Rogers, Clark and Evans[26] of a distinct difference in habitat that made the differentiation of *B. coli* from *B. aerogenes* of capital significance.

Rogers and his associates showed that the true *B. coli* is characteristic of bovine feces while the *B. aerogenes* type is the characteristic form found on grains. Of 150 strains from bovine feces only one was of the high ratio type against 151 out of 166 grain cultures.

[21] *Jour. Wash. Acad. Sci.*, 1915, vol. v, p. 609; also *Jour. of Infect. Dis.*, 1915, vol. xvii, p. 109.

[22] *Jour. of Bact.*, 1916, vol. i, p. 81.

[23] *Proc. Roy. Soc.* (B), 1905–1906, vol. lxxvii, p. 399.

[24] *Proc. Roy. Soc.*, 1905–1906, vol. lxxvii, p. 424.

[25] *Proc. Roy. Soc.* (B), 1911–1912, vol. lxxxiv, p. 492; also *Proc. Roy. Soc.*, 1912, vol. lxxxv, p. 73; also *Jour. of Physiol.*, 1911, vol. xlvii, p. 332.

[26] *Jour. of Infect. Dis.*, 1914, vol. xv, p. 100; also *Jour. of Infect. Dis.*, 1915, vol. xvii, p. 137.

More recently Rogers, Clark and Lubs[27] have reported that 107 out of 113 human fecal strains were of the low ratio type. Rogers[28] found in a study of 137 cultures isolated from water that the *B. coli* type was found occasionally in springs in which there was no

TABLE 4

Hydrogen ion concentration produced by B. coli and B. aerogenes in glucose pepton broth

CULTURE	B. COLI P_H^+ VALUE AFTER						
	2 hours	4 hours	8 hours	1 day	2 days	4 days	7 days
17	7.3	7.2	6.1	4.8	4.7	4.5	4.6
19	7.2	6.8	5.6	4.8	4.7	4.5	4.8
44	7.3	7.2	6.2+	4.8	4.8	4.8	5.0
45	7.0	7.0	6.0	4.8	4.8	4.5	4.6
47	7.3	7.2	6.2+	5.0	5.0	4.4	4.8
52	7.3	7.0	6.0	4.8	4.3	4.5	4.5
78	7.2	7.2	6.1	4.8	5.0	5.0	4.9
95	7.3	7.1	6.0	4.7	4.8	4.6	4.5
104	7.2	7.0	6.1	4.7	4.3	4.4	4.8
125	7.1	7.1	6.0	4.8	4.3	4.7	4.6

CULTURE	B. AEROGENES P_H^+ VALUE AFTER						
	2 hours	4 hours	8 hours	1 day	2 days	4 days	7 days
23	7.3	7.2	6.0	5.8	6.2	7.2	7.2
24	7.2	6.9	5.6	5.5	6.2	7.2	7.1
123	7.3	7.1	5.8	5.7	6.2	6.6	6.8
136	7.2	6.6	6.0	5.7	7.0	7.2	7.2
233	7.3	7.2	6.2+	5.8	6.2	7.2	7.2
240	7.2	7.2	5.6	5.6	6.6	6.5	6.6
369	7.2	7.0	5.6	6.0	6.6	7.0	6.8
454	7.3	7.2	6.2+	5.1	6.0	6.4	7.1
529	7.2	6.8	5.5	5.8	6.6	6.5	6.4
583	7.3	7.2	5.8	5.7	6.9	7.2	7.2

evident source of contamination but was especially abundant in sewage polluted rivers, while the *B. aerogenes* type was found in waters of all sorts. Johnson[29] reports that of 363 colon group

[27] *Jour. of Bact.*, 1916, vol. i, p. 82.
[28] *Jour. of Bact.*, 1916, vol. i, p. 82.
[29] *Jour. of Bact.*, 1916, vol. i, p. 96.

organisms from soil, 261 were of the *B. aerogenes* type and of these 219 gave the Voges-Proskauer reaction.

The general correlation of the two different types of carbohydrate fermentation with indol fermentation, saccharose fermentatation, dulcite fermentation and the Voges-Proskauer reaction appear to explain many of the results obtained by MacConkey, Houston and Clemesha in regard to the special significance of "typical" *B. coli.* Since, however, there are bacilli of the *B. coli* fermentative type which ferment saccharose and fail to ferment dulcite and some of the *B. aerogenes* type which form indol the results of these earlier observers were conflicting and unsatisfactory. The radical difference in fermentative power is evidently the most fundamental of all the lines of demarcation within the colon group, and a study of this reaction promises to be of great value in sanitary water analysis.

The simplest differential test for the two types is the hydrogen ion concentration in glucose pepton broth. The exact concentration will of course vary with the composition of the medium and the time and temperature of incubation. In the writer's own work he has used a broth made up with 0.5 per cent glucose, 0.5 K_2HPO_4 and 1.0 per cent Witte's Pepton. Clark and Lubs[30] recommend 0.5 per cent pepton instead of 1.0 per cent. The cultures were incubated in the writer's studies at 30° but he sees no reason why a 37° incubation should not be equally satisfactory for routine sanitary purposes. At the end of 48 hours the cultures should be tested by the addition of a few drops of a methyl red solution (0.1 gram methyl red dissolved in 300 cc. alcohol, and diluted to 500 cc. with distilled water). At a hydrogen ion concentration of about 5.8 $\left(P\frac{+}{H}\text{ value}\right)$ methyl red changes from red to yellow so that, as may be inferred from the data cited on in Table 4, the *B. coli* type will show a brilliant red coloration while the *B. aerogenes* cultures will be yellow.

Only extensive studies of the distribution of bacteria of these two types in waters of different quality and a correlation of the results with those of sanitary inspection will give us a safe basis for an interpretation of the respective significance of the *B. aerogenes* and *B. coli* types. The errors which we have all made in the application of the colon test in the past should be a warning against hasty

[30] *Jour. of Infect. Dis.*, 1915, vol. xvii, p. 160.

generalization. Yet the results already obtained are sufficiently promising to suggest that we have at last perhaps a test which may really differentiate fecal colon bacilli from related types found in soil and on grains and grasses. The writer trusts that the bacteriologists in water works laboratories will take advantage of their unusual opportunities for accumulating data in regard to this point by applying the simple methyl red test outlined above to subcultures from confirmatory Endo or lactose agar plates in as large a proportion of colon isolations as possible. We know today that the whole group of aerobic gas formers, *B. coli* and *B. aerogenes* alike, are not found in large numbers in waters of high quality; but if *B. coli* proves indeed to be a purely fecal form and *B. aerogenes* a saprophyte the precision of our tests will be greatly increased and an accurate interpretation vastly simplified.

DISCUSSION

DR. W. P. MASON: As set forth in the title of this paper it deals with an indicator of water pollution. From a bacteriological standpoint all that Professor Winslow has had to say is doubtless very complete and very well done, and we are of necessity under obligations to him; but from a practical standpoint the speaker would hardly like to throw overboard the old presumptive test, and tie up to anything very new until that new procedure has been pretty thoroughly thrashed out; in dealing with water matters he likes to get all types of facts before him, bacteriological, chemical and, above all, the sanitary survey. Now, a word with reference to something that happened the day before yesterday. The speaker was asked to look into and pass upon a question of damage to a deep well, a well having a depth of over 100 feet, and he sent a trustworthy member of his staff to take a sample and make a sanitary survey. The sample was taken and the sanitary survey reported as excellent. The house was on the top of a ridge, and the well was 100 feet deep, drilled in shale rock. The house was sewered, with delivery to a cesspool, and the cesspool to which the sewer ran was a long distance away. There were no barns nearby but there was a garage. In making an examination of the water by the presumptive test gas was found to a slight extent, one tube in ten. However, the chlorine ran high; so did the nitrates and there were

some nitrites as well. The man who took the sample was interrogated and nothing was found the matter with his sanitary survey. On a personal investigation the premises were found as reported, but, closer inspection disclosed behind the garage a very small privy, described by the owner as "nothing in particular," adding that "it had not been used for a long time." However, it had been used, probably inside of half a day. It had been in regular use about once a day, by probably one person only, and it was within ten feet of the mouth of the well. This is but another instance showing how valuable it is to secure all forms of information bearing upon the case when asked to pass judgment upon the quality of a water—laboratory data alone may be misleading, not with any desire to belittle the value of bacteriological or chemical examinations. We want all the light that can be given but if but one form of information is available, the speaker would rather have the sanitary survey than all the rest of them put together. No result of bacteriological examination, or chemical results should be taken as material; the sanitary survey is enough. Nor should it be questioned whether the party using the privy happens to be capable of giving a disease so far as the result on the worth in the water is concerned. To repeat, it is better not to tie up to any one particular test, but to make a bacteriological and a chemical examination, to take both into consideration, and to put the sanitary survey above them all.

DR. FRANK E. HALE: The speaker has been very much interested in Mr. Winslow's paper, particularly the question of methods, and cannot help making a few remarks upon three points brought up by Mr. Winslow. In the first place, as to the classification of *B. coli*, the speaker cannot agree with Mr. Winslow that the present classification of standard methods is not a useful one. That classification is a real classification and is useful in distinguishing varieties that are real and distinctive. All the forms are of fecal origin, many originally were isolated from diseased conditions. Vaccine made from them cured the conditions when the specific form had been isolated. A classification which enables one to do this cannot be said to have outgrown its usefulness. Again all of the varieties were kept for years and retained their original characteristics. The proposed classification is on broader lines and less specific in detail. It merely divides into groups those now contained in the present classification, and could not possibly show a wide adapta-

bility. To introduce into routine work additional tests to the presumptive test, so as to limit significance to certain groups of the present classification, is an entirely different and separate question, and offers no argument for discarding the present classification.

No advance will be made until we are able to distinguish between animal and human *B. coli* varieties, if that ever be possible. Croton water has shown tests for *B. coli* in 0.1 cc. daily for a month, when inspection disclosed no probability of human pollution. The direct test for *B. typhii* from the bile tubes then becomes important.

In the second place, as to the use of lactose bile versus lactose broth, even if the presumptive test is not alone relied upon, lactose broth was found wanting long before bile was discovered. Why should we now go backward? Since the Committee on the Revision of Standard Methods has advocated the use of lactose broth we have again made at Mt. Prospect Laboratory a series of comparisons with the lactose bile and lactose broth, confirming by litmus lactose agar. The broth was made as recommended by the committee; the bile was 5 per cent as recently recommended from this laboratory. The results were all in favor of bile, quicker gas formation, gas in larger amounts, and less *B. Welchii* forms. In one day the results with bile were practically equal to those obtained in two days with the broth. Very few bile tubes show less than 10 per cent of gas, whereas many broth tubes show small amounts of gas, and the larger amounts of gas in the broth were produced by *B. Welchii* forms. It is not safe to trust small amounts of gas, since such may be due to inversion of lactose to dextrose in sterilizing. Possibly it would be well to lower the presumptive requirements with lactose bile to plus 10 per cent gas and make corroborative tests when conditions call for it. This would retain the presumptive test where permissible and allow of direct test for *B. typhii* from the bile tube on Hesse or other media. Most previous comparative work has been with 10 per cent bile, and frequently improper bile has been used. Desiccated bile of the right quality may now be obtained.

In the third place, as to *B. Welchii* forms, the speaker believes they are of direct sanitary significance. They are associated in large numbers with pernicious anaemia and dozens of other diseases. The speaker does not believe the percentage of these forms in the presumptive bile test is commonly as high as has recently been stated. Creel was dealing with special conditions, tanks on

railroad trains where opportunity was excellent for accumulating spores. If the ordinary surface water contained a high percentage of *B. Welchii* spores chlorination could not show the percentage removal of *B. coli* by the presumptive test that is common experience. Removal of 90 per cent and more is not uncommon in New York City supplies, and this is probably not exceptional. It may be, however, that the introduction of chlorination has increased somewhat the percentage of *B. Welchii* forms found today over that of previous experience. This has been found to be true recently in comparing chlorinated with unchlorinated waters.

MR. C.-E. A. WINSLOW: With reference to Dr. Hale's comparison of the lactose bile and the lactose broth tests, it may be that conditions are different in New York waters. We have taken our data from results obtained in various places; different laboratories in various parts of the country. Of course Dr. Hale's tests show the value of the presumptive test in New York waters, but it does not work out the same way in the case of other waters. The *B. Welchii* group for some reason is apparently not abundant in New York water; but it is very abundant in a great many other waters in different parts of the country. The speaker does not see why the confirmatory test should not be retained and used in places where the *B. Welchii* group is not abundant.

He cannot feel that soil washings and from manured fields are so negligible as Dr. Hale believes, and is inclined to take the stand that Dr. Mason did when he had the experience of finding the privy over the well. We have sufficient data from all over the country of serious disease caused by washings from the surface of the soil, to make us feel that no water supply is ever safe if exposed to such sources of contamination, whether they have sewers emptying into them or not. There are several cases in point where there was doubt as to the source of the colon bacilli, whether from privies or manure piles, or what not, but where the danger from an epidemic of typhoid was always present until the waters were chlorinated. The speaker is naturally in accord with what Dr. Mason said about the sanitary inspection. That is the most essential thing, one of the most essential means of determination of the source of contamination, but there are many cases where the sanitary inspection does not help; where the cause is obscure.

DR. FRANK E. HALE: It is certain, however, that we get better results with the bile test, and from our experience in New York we believe that it is surer.

MR. DANIEL D. JACKSON: Unfortunately the speaker has not done any laboratory work along this line in the past three years, and has no new results in connection with it, aside from what has already been discussed and printed, but would like to add this word concerning the great value of sanitary inspection as related to analysis. He had occasion, only a short time ago, to examine a water supply which was very high in *B. coli*, and also in chlorides. This water supply had been condemned by the state, one of the southern states, without what the speaker considered an adequate examination as to sanitary conditions. Any examination into the condition, from the analysis alone, would have undoubtedly led anyone to condemn it. Sanitary inspection, however, showed that on one of the series of wells the chlorine was considerably beyond what would be found in any sewage. Therefore it did not come from the sewage. There was a natural salt pocket at one end of the well system, as these wells were on the edge of the coastal plane. By discontinuing half a dozen of the wells on one end of the line and carrying the well line to the other side, the wells could be relocated so as to contain the natural amount of salt.

Now, as to the *B. coli*. It was necessary to get up at four o'clock in the morning to find out where the *B. coli* came from, when, just as the sunrise began, was revealed some four or five turkey buzzards on top of the collecting or receiving chambers where the water was being pumped from each well by an air lift. The turkey buzzards were not particular which way they were facing. There was a collection of some 25 wells, and the receiving chamber of each gave tests for *B. coli* which were positive in 1 cc. This would ordinarily condemn them, but, by properly covering these receiving chambers, the coli content was reduced to zero. Now, it is not right to condemn a property without a proper sanitary inspection. It is quite right to report against it, but the source should be inspected to see if it can be fixed up rather than, as in this case, to condemn $200,000 worth of property.

DR. W. P. MASON: A case entirely similar to that came under the speaker's observation. The turkey buzzards were not in evi-

dence but a flock of small birds were. It could not make any difference which way they turned, as they were so short. The deep well water which flowed into an open funnel like receiver caught the pollution.

MR. CHESTER G. WIGLEY: The coli determination with lactose bile is a very valuable aid in the work of the state department of health, especially in field operations. Experience with it shows that it is the best self contained process that can be used in the field. For that reason the department has hesitated to stop the use of it altogether; that is, where a man is limited by the amount of material that he can carry and by his very small laboratory facilities, because in field work the opportunities for confirmation are not very good. The bile figures have given very valuable information fairly indicative of conditions found by sanitary surveys while the broth indications were not nearly as clear, or as satisfactory. There is another consideration in reference to the proposal of the new standard for coli determination, and that is the fact that it is unfortunate that just about the time we begin to get some fair understanding of one test a new process is proposed, the limitations of which have not yet been worked out. It may be that the test which Professor Winslow suggests would have certain advantages, if its use under particular or peculiar conditions were tested out so that it would not mean that we would run into more complications. In many ways its adoption would cause difficulties to some of us who work in the field, because it would mean that we would of necessity be compelled to carry along supplies for both methods, for the purposes of comparison, before we could feel perfectly safe in basing our opinions upon the result of new methods.

MR. MAYO TOLMAN: The speaker has been especially interested in Professor Winslow's paper, as several years ago he made a study of the pollution of the Assabet River between the towns of Hudson and Concord, Massachusetts, and at that time, being curious to see how the species of colon bacilli found in polluted water would compare with those isolated from feces by MacConkey, Winslow, Walker and others, made a detailed study of quite a number of samples. For the purpose of determining the species of the colon bacillus present, a pure culture was isolated by the streak method, on blood serum from that tube of lactose bile of the greatest pollu-

tion, showing between 20 per cent and 80 per cent gas production. The fermenting power of this culture toward dextrose lactose, dulcite, saccharose, mannite and raffinose was determined, as was also the motility of the organism, reduction of nitrates, production of indol, liquefaction of gelatin and coagulation of milk and the species of colon thus determined. The results of these determinations of twenty-four samples, which constituted one run, are appended in the following table:

DILUTION	PER CENT GAS	NUMBER OF COLI PER CC.	DEXTROSE	LACTOSE	DULCITE	SACCHAROSE	MANNITE	RAFFINOSE	MOTILITY	INDOL	NITRATE REDUCTION	LIQUEFACTION OF GELATIN	COAGULATION OF MILK	SPECIES
1.0	....	....	+	+	−	−	+	−	+	+	+	−	+	B. acidi-lactici
0.001	....	....	+	+	−	+	+	+	+	−	+	−	+	B. aerogenes
0.01	....	....	+	+	−	+	+	+	+	−	+	−	+	B. aerogenes.
1.0	....	....	+	+	−	−	+	+	+	+	+	−	+	B. acidi-lactici
0.1	80	60	+	+	−	+	−+	+	−	+	+	−	+	B. aerogenes
1.0	70	6	+	+	−	−	+	+	+	+	+	−	+	B. acidi-lactici
0.1	90	2000	+	+	−	−	+	+	+	+	+	−	+	B. acidi-lactici
1.0	70	2	+	+	−	−	+	+	+	+	+	−	+	B. acidi-lactici
0.01	95	2	+	+	−	+	+	+	−	+	+	−	+	B. aerogenes
1.0	70	80	+	+	−	+	+	−+	+	−	+	−	+	B. aerogenes
0.1	80	20	+	+	−	+	+	+	−	+	+	−	+	B. aerogenes
0.1	70	80	+	+	−	+	+	+	+	−	+	−	+	B. aerogenes
0.01	100	400	+	+	−	+	+	+	+	−	+	−	+	B. aerogenes
0.1	50	100	+	+	+	+	+	+	+	−	+	−	+	B. communior
0.1	85	100	+	+	−	+	+	−	−	−	+	−	+	B. aerogenes
0.1	60	20	+	+	−	+	−	+	−	+	+	−	+	B. aerogenes
1.0	100	6	+	+	−	+	+	+	−	+	+	−	+	B. aerogenes
0.1	80	40	+	+	−	−	+	−	+	+	+	−	+	B. acidi-lactici
0.1	50	100	+	+	−	+	+	+	−	+	+	−	+	B. aerogenes
0.1	80	40	+	+	−	−	+	+	+	+	+	−	+	B. acidi-lactici
0.1	95	40	+	+	−	−	+	+	−	+	+	−	+	B. acidi-lactici
1.0	80	10	+	+	+	−	+	+	+	−	+	−	+	B. communior
0.1	60	40	+	+	−	−−	+	−	−	−	+	−	+	B. acidi-lactici
0.01	85	400	+	+	+	−	+	+	+	+	+	−	+	B. communior

The three samples that gave bacillus communior were taken from points where the river water was kept at a high temperature; in one case by the hot wastes from a tannery, in the second by the discharge of hot condenser water, while the third was taken from the effluent drain of a slow sand filter handling sewage. In other

words, they were taken at points where the temperature conditions more closely approximated that of feces. All the other samples, namely those which show the acidi-lactici and aerogenes type, were taken at points where the river passed through open fields some distance from any sewer. Not knowing the work done by Professor Winslow, Dr. Houston, and others, the speaker felt that he had possibly discovered something new and useful, and continued the work with a view of determining whether or not the addition of the dulcit fermentation test to the ordinary routine procedure for determining the presence of the colon group might not give us valuable additional information as to the probable sources of the organisms encountered.

The speaker is very glad to see that his conclusions were along the right line and that there is a similar but less expensive and more practicable method of determining, with reasonable certainty, whether the colon organism as found in water is of fecal origin or not.

MR. C.-E. A. WINSLOW: The speaker wants to make his position entirely clear; he does not think we are in a position to interpret the difference between the types of *B. coli* absolutely, but the work that has been done has been sufficiently promising to warrant its being further looked into. The condemnation of waters on the basis of the lactose bile presumptive test, however, whether they have any trace of the true colon group in them or not, is most unfair; it has led to serious injustice to many water works throughout the country. The accuracy of the tests as applied to certain waters like the Croton watershed is not doubted, but it has been misleading to apply the same tests to other water supplies, which were of high quality. Indiscriminate condemnation of water supplies ought to be stopped because they give the lactose bile presumptive test, unless we are certain they are confirmed by other tests for the group of aerobic bacilli which do not form spores.